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Diffusion of Some Nucleotides into Outer Medium from Non-Irradiated and Gamma-Irradiated *Euglena* Cells

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When non-irradiated *Euglena* cells were suspended into distilled water and then were incubated for several hours, some ultraviolet absorbing substances diffused out of the cells into distilled water. The amount of these substances increased according to cell concentration and to incubation temperature. When the cells were exposed to 0.5, 1, and 2×10^5 r gamma-rays respectively and then were incubated as above, similar ultraviolet absorbing substances diffused in large quantity out of the irradiated cells. The total amount of these diffused-out substances was rather small as compared with the total amount of acid soluble ultraviolet absorbing substances in the cells, that is, about 10.1% during a 3-hour incubation period after irradiation. It increased according to the irradiation dosage given. When these diffused-out substances from both non-irradiated and irradiated cells were investigated with Dowex-1-formate column, it was found that they contained some nucleosides and nucleotides, and also that they changed in composition before and after the irradiation. That is, UMP,** GMP, and UDP were recognized only after the irradiation. "R-substance", AMP, and ATP markedly increased in quantity after the irradiation. Contrary to this, some uncleosides, GTP, and UX decreased in amount after the irradiation.

INTRODUCTION

In the course of investigation on ionizing radiation damage of RNA level in *Euglena* cells, it was found that a decreased level of intracellular nucleotides had some connection with the RNA level of irradiated cells.[1] Then we have investigated the change of intracellular mononucleotide composition[2] of the irradiated cells. On the way of this mononucleotide investigation, a noticeable fact that some ultraviolet absorbing substances diffused out of both non-irradiated and irradiated cells was observed. By irradiation, the amount of these diffused-out substances was generally increased. This phenomenon may also be a cause of the decreased RNA level by irradiation. This paper deals with some qualitative and quantitative changes of these diffused-out substances.

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** Abbreviation used in this paper: RNA; ribonucleic acid, AMP and APT; adenosine mono- and tri-phosphate, GMP and GTP; guanosine mon- and tri-phosphate, CMP; cytidine monophosphate, UMP, UDP, and UTP; uridine mono-, di-, and tri-phosphate, UX; a kind of uridine compounds, DPN; diphospho-pyridine nucleoside, R-substance; the substance which is eluted from the Dowex column with distilled water. λ_{max}; wavelength of absorption maximum, λ_{min}; wavelength of absorption minimum.

MATERIAL AND METHODS

Material and Irradiation

Two kinds of *Euglena* cells (*Euglena gracilis var. bacillaris*), logarithmic phase cells and stationary phase cells, were used as the starting material. The cells were harvested centrifugally from the cultures of two different growth phases; the logarithmic phase cells were obtained by incubation for 6 days in a pepton nutrient solution[2] and the stationary phase ones, by incubation for 20 days in the same solution. Then the cells were washed thoroughly with distilled water. After the washing, about 5.7×10^9 cells were suspended in 900 ml of distilled water respectively. This cell suspension was divided into two aliquots of 120 ml and 780 ml. The 120 ml aliquot was used for Experiment I. In this experiment, the effects of cell population and incubation temperature on the diffusion of the ultraviolet absorbing substances from the cells were investigated. Cell suspensions of different cell populations were prepared by diluting the original suspension with water or by concentrating the cells centrifugally. They were incubated at 30°C (Fig. 1), or at 2, 15, and 30°C, respectively (Fig. 2). The remaining 780 ml aliquot was used for Experiment II. In this experiment, the relationship between irradiation dosage and diffusion amount of the ultraviolet absorbing substances, and the change of ion exchange chromatographical patterns of the substances before and after the irradiation were investigated. This aliquot was further divided equally into 6. Each aliquot was put into a polyethylen capsule. Three capsules among them were exposed to the gamma rays of 0.5, 1, and 2×10^5 r, respectively. The dose rate of the irradiation facility used was 1.09×10^5 r/hr.

The rest three capsules were used as the control corresponding to each irradiation experiment with the different dosage mentioned above. The irradiation was carried out with the Co-60 gamma-ray irradiation facility of the Institute for Chemical Research of Kyoto University. Immediately after the irradiation, each 10 ml of the irradiated and the non-irradiated cell suspensions was transferred into a frask to investigate the relationship between irradiation dosage and diffusion amount of the ultraviolet absorbing substances. The remaining 120 ml of each irradiated and non-irradiated cell suspension was used for ion exchange chromatography. After the transfer of the 10 ml cell suspension, all the irradiated and the non-irradiated cell suspensions were incubated at 30°C until they were used for each experiment. After the incubation, in both Experiments I and II, the cells were eliminated from each cell suspension by centrifugation at 3000 rpm for 3 min. at 0°C. This supernatant was used as the material to be studied in the present study. No dead cell was recognized in both the irradiated and the non-irradiated cell suspensions during the various incubation periods, except a 72-hour incubation of the irradiated cells. In the case of this 72-hour incubation, the percentage of dead cells found was to be about 2 %.

Absorption Spectroscopy

All the absorption spectra and optical density measurments were carried out with a Hitachi EPU-2 spectrophotometer.

Ion exchange chromatography: Each 120 ml of the supernatant mentioned above, which was obtained from both the non-irradiated and the irradiated cell suspensions incubated for 6 hours at 30°C after the irradiation, was concentrated to 15 ml with a Kyowa RL-200S freeze-drying apparatus. For chromatography, Dowex 1-X10, chloride form (200-400 mesh), was converted into formate form by the procedure of Hurlbert *et al.*[3] A resin bed 1 cm in diameter and about 12 cm in height was used. After the concentrated supernatant mentioned above was absorbed to the column, the column was washed with distilled water, until the absorbancy at 260 mu of the effluent was less than 0.01 per ml. The nucleotides were eluted with a modified formic acid and ammonium formate elution procedure of Cohn and Volkin.[4] Five ml elutes were collected with constant flow of about 8 drops per min. at 10°C. The absorbancy was read at 260 and 275 mu. The identification of the nucleotides was made; (a) by comparison of the elution patterns obtained with those described in the literature,[3~5] (b) by comparison of the elution sequence with that obtained with commercial nucleotides, and (c) by comparison of the absorption spectra of efflutes with those of commercial nucleotides.[6] The relative amount of effluent in a given fraction was determined by total absorbancy at 260 mu of the fraction. The correction for the background absorbancy of the eluting solution was made by deduction of the absorbancy of eluting solution themselves from that of the elute.

Little difference between the logarithmic phase cells and the stationary phase cells used in the present study was observed with regard to the diffusion-out of the ultraviolet absorbing substances. Moreover, the results obtained with ion exchange chromatography were almost similar to one another among the substances coming from the materials subjected to these three different irradiation dosages mentioned above. Consequently, with regard to the diffusion amount of the ultraviolet absorbing substances, only the results obtained with the logarithmic phase cells, and with regard to the ion exchange chromatography, only the results obtained with the logarithmic phase cells exposed to 1×10^5 r gamma rays are described in this paper.

RESULTS

Experiment I. Diffusion of Ultraviolet Absorbing Substances from Normal Cells into Outer Medium

1) **Effect of cell population on diffusion.** From the original cell suspension containing about 7.4×10^8 cells, 5 cell suspensions of different cell population were prepared; 2.8×10^5 cells/ml, 1.0×10^6 cells/ml, 2.5×10^6 cells/ml, 1.1×10^7 cells/ml, and 2.5×10^7 cells/ml. The volume of each cell suspension was 10 ml. These cell suspensions were incubated in separate flasks for 40 hours at 30°C. The amount of diffused ultraviolet absorbing substances found in each outer medium was estimated by measuring absorbancy at 260 mu of the outer medium serveral times during the incubation period. As shown in Fig. 1, the total amount of the diffused-out substances increased according to cell concentration and to incubation time.

2) **Effect of incubation temperature on diffusion.** Three 10 ml aliquots of

cell suspension (1.0×10^6 cells/ml) where incubated for 40 hours at 2, 15, and 30°C, respectively. Then the relationship between incubation temperature and amount of the diffused-out substances was investigated. The result obtained is shown in Fig. 2. This shows that the diffusion amount of the ultraviolet absorbing substances increased according to incubation temperature.

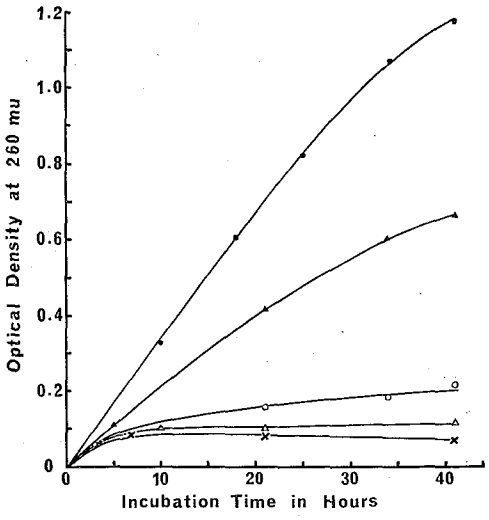

Fig. 1. Relationship between cell concentration and diffusion rate of ultraviolet absorbing substances.
2.8×10^5 cells/ml : (—×—),
1.0×10^6 cells/ml : (—△—),
2.5×10^6 cells/ml : (—○—),
1.1×10^7 cells/ml : (—▲—),
2.5×10^7 cells/ml : (—●—).

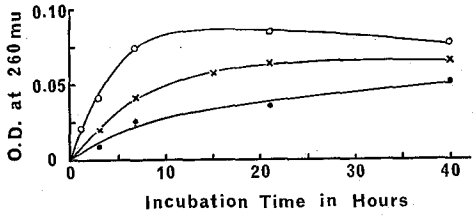

Fig. 2. Relationship between incubation temperature and diffusion rate of ultraviolet absorbing substances.
Cell concentration : 2.8×10^5 cells/ml
Incubation temperature : 2°C ; (—●—), 15°C ; (—×—), and 30°C ; (—○—).

Experiment II. Effects of Irradiation on the Diffusion of Ultraviolet Absorbing Substances

1) Absorption spectra of the substances diffused out of non-irradiated and irradiated cells. When 10 ml of the non-irradiated cell suspension containing about 6.3×10^7 cells was incubated at 30°C, no ultraviolet absorbing substance was found in the outer medium during the first 1 or 2 hours, but some ultraviolet absorbing substances were found to diffuse out of these cells during 6 hours of incubation. The absorption spectrum of these diffused-out substances showed λ_{max} at 260 mu and λ_{min} at 240 mu (Fig. 3 ; -o-).

When the similar cell suspension was exposed to 1×10^5 r gamma rays and then incubated for 6 hours at 30°C, the ultraviolet absorbing substances diffused in larger quantity by about 31 % than the case of the non-irradiated cells. The absorption spectrum of these substances had λ_{max} at 257 mu and λ_{min} at 240 mu (Fig. 3 ; -•-). A similar absorption spectrum was also obtained with the diffused-out substances originating from the cells subjected to either 0.5 or 2×10^5 r irradiation.

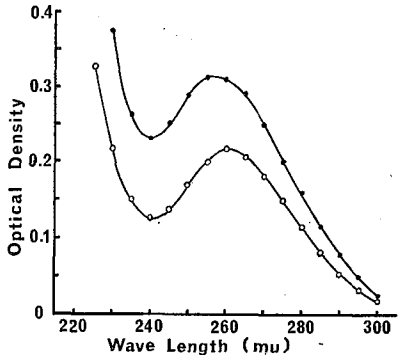

Fig. 3. Absorption spectra of the ultraviolet absorbing substances diffused out of non-irradiated and gamma-irradiated cells.
Non-irradiated : (—○—), Gamma-irradiated : (—●—).

2) **Irradiation dosage and diffusion rate of the ultraviolet absorbing substances.** From the non-irradiated and the irradiated cell suspension, every 10 ml aliquot containing about 6.3×10^7 cells was transferred into a flask and incubated respectively for 3, 24, and 72 hours at 30°C to investigate the relationship between the irradiation dosage of 0.5, 1, and 2×10^5 r and the diffused-out amounts of the ultraviolet absorbing substances. The amounts were determined by measuring absorbancies at 260 mu of the 10 ml aliquot of supernatants, and they are given in Fig. 4. In the cases of the non-irradiated cells, the "total acid soluble fraction" was extracted from the cells with 10% perchloric acid for one hour at 0°C, and the absorbancy at 260 mu of this fraction was determined to estimate the amounts of the diffused-out substances in terms of the absorbancy of this fraction. The amount of the substances diffused out during a 3-hour incubation period was about 10.1 %, that diffused out during 24 hours about 24.3 % and that during 72 hours about 53.4 %. In the cases of the irradiated cell suspensions, the amount

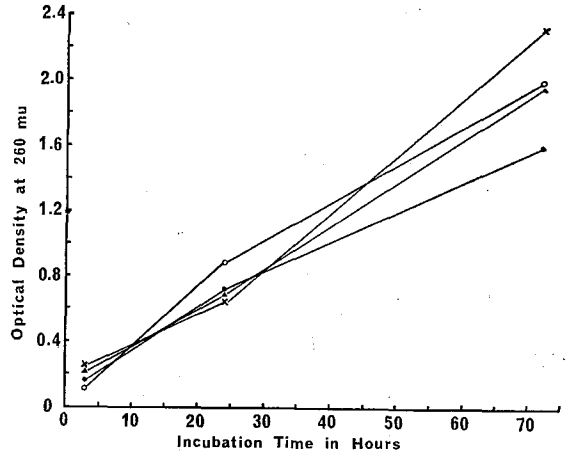

Fig. 4. Relationship between irradiation dosage and diffusion rate of ultraviolet absorbing substances.
0.5×10^5r : (—×—), 1.0×10^5r : (—▲—)
2.0×10^5r : (—●—), Non-irradiated : (—○—)

of the substances generally increased according to the irradiation dosage and to the incubation time (Fig. 4). Comparing the cases of the non-irradiated with those of the irradiated cell suspension, the diffused amount was smaller in the former cases in the first 3-hour incubation period after the irradiation, but larger in a 24 hour incubation period than in the latter cases. In a 72-hour incubation period the diffused-out amount was small in the 0.5×10^5 r irradiated cell suspension, medium in the non-irradiated and the 1×10^5 r irradiated ones, and large in the 2×10^5 r irradiated one. Although the amounts of diffused ultraviolet absorbing substances of both the non-irradiated and the 1×10^5 r irradiated cell suspensions incubated for 72 hours were almost of same value, some differences in nucleotide composition was found by ion exchange chromatography in this case. Such a phenomenon will be described later in detail (Fig. 5).

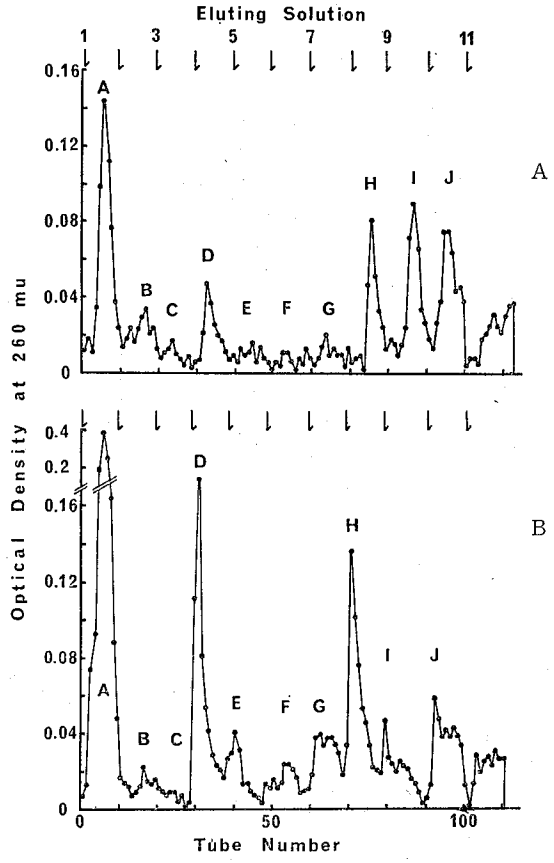

Fig. 5. Chromatographic patterns of the nucleotides diffused out of non-irradiated and gamma-irradiated cells.

Eluting solution : (1) distilled water, (2) 0.01M ammonium formate (Af), (3) 0.02M Af, (4) 0.15N formic acid (F), (5) 0.05M Af+0.01NF, (6) 0.1M Af+0.1NF, (7) 0.5M Af+0.1NF, (8) 2.5M Af, (9) 2.5M Af+0.5NF, (10) 2.5M Af+2NF, (11) 2.5M Af+4NF. Volume of each eluting solution : 50ml (A) : non-irradiated, (B) : gamma-irradiated.

3) **Nucleotide composition of the substances diffused out of non-irradiated cells.** After the non-irradiated cell suspension containing about 7.6×10^8 cells in 120 ml of distilled water was incubated for 6 hours at 30°C, the cells were eliminated by centrifugation and the resulting supernatant containing the ultraviolet absorbing substances was concentrated by freeze-drying. The concentrated ultraviolet absorbing substances were absorbed to the Dowex-1-formate column and eluted with stepwisely increasing concentration of formic acid and ammonium formate (Explanation of Fig. 5). During the incubation period mentioned above, the total cell number did not increase at all in the present experiment. As shown in Fig. 5, A, the ultraviolet absorbing substances contained some mononucleotides, and they were separated fairly well with every eluting solution. For the convenience's shake, all the fractions obtained in the present experiment are designated by A, B, C,⋯.

Fraction A eluted with distilled water had no absorption in ultraviolet region as it was eluted, but when concentrated by freeze-drying, showed an absorption maximum at about 250 mu. Therefore, it seems probable that some purine and pyrimidine bases or nucleosides may exist in this fraction as reported in E. coli[7] and in yeast cells.[4] But this fraction was not analysed further in the present experiment. Fractions B and C had an absorption maximum in the region near 270 to 275 mu. According to Cohn and Volkin,[4] these two fractions contain some nucleotides. But in the present experiment they could not be identified, because they were found only is small quantity. Fraction D, which had λ_{max} at about 270 mu contained CMP and AMP(a). The ratio of CMP to AMP(a) was about 1 : 0.5. This quantitative ratio was determined by a rechromatographical separation with the same Dowex-1-formate column as above in a cold room at 3°C. Judging from the elution sequence,[4,5] fraction E correspond to AMP (b) and UMP, fraction F to GMP, and fraction G to UDP. Fraction H which had λ_{max} at 260 mu contained ATP. Fraction I which had λ_{max} at about 250 mu contained GTP. Fraction J which had λ_{max} at about 260 mu may contain some kind of uridine compound (UX).[3]

4) **Nucleotide composition of the substances diffused out of irradiated cells.**

After the cell suspension containing about 7.6×10^8 cells in 120 ml of distilled water was exposed to gamma rays of 1×10^5 r and then incubated for 6 hours at 30°C, the ultraviolet absorbing substances diffused out of the cells were prepared as mentioned in the previous paragraph. They were absorbed to the Dowex column and eluted stepwisely with the eluting solution. The nucleotide composition in this case is shown in Fig. 5, B. As some quantitative changes in nucleotide composition were markely recognized after the irradiation, these change are mainly described in the following description.

After the irradiation, fraction A or "R-substance" increased about 2.9 fold in quantity, as judged by total absorbancy at 260 mu of the fraction than in the case of the non-irradiated cells. This fraction had λ_{max} at 250 mu in the fraction as it was eluted. Therefore, it may be probable that some purine and pyrimidine bases or nucleosides[6] diffused out of the irradiated cells in a considerably large

quantity into this fraction. Fraction B decreased in quantity by about 47.3%, and fraction C by about 54.2% after the irradiation. Contrary to this, there are some fractions which showed an increse in quantity after the irradiation. For example, fraction D increase about 2.7 fold in quantity after the irradiation. This fraction had λ_{max} at 260 mu, which diffused from that in the case of the non-irradiated cells. This difference in absorption maximum is due to an increase in amount of AMP, which has λ_{max} at 260 mu, because the ratio of CMP and AMP in this fraction D was about 1 : 3.4, while any quantitative change of CMP was hardly recognized before and after the irradiation. Fraction E which had λ_{max} at 260 mu increased about 2.1 fold in quantity after the irradiation, fraction F which had λ_{max} at 260 mu about 2.2 fold, fraction G which had λ_{max} at 272 mu about 3.6 fold, and fraction H which had λ_{max} at 260 mu about 1.8 fold, respectively. Fraction I decreased in quantity by about 29.1%, and Fraction J by about 9.3% after the irradiation.

Among all the fractions mentioned above, 3 fractions ; A, D, and H, markedly increased in quantity after the irradiation in comparison with the other fractions. Moreover, although some fractions decreased in quantity after the irradiation, most effluates in the case of the irradiated cells were more optically denser than those in the case of the non-irradiated ones. Accordingly, the total amount of all the fractions increased by about 1.6 fold after the irradiation.

DISCUSSION AND CONCLUSION

Some workers reported that nucleic acids were decomposed *in vivo* and *in vitro* into some low molecular substances by an ionizing radiation.[8~10] But unfortunately little data are available for the diffusion-out of some substances decomposed by ionizing irradiation from irradiated cells, except some reports by Beyer *et al.*[11] and Cole *et al.*[12] Beyer *et al.* have reported that some uncleotides, such as DPN, AMP, ATP etc. are released from the mitochondria of yeast cells by ultraviolet irradiation, and Cole *et al.* have found that leaking of soluble deoxypolynucleotide from spleen tissue is increased by x-ray irradiation. In the present study also, some phenomena similar to these were observed. From the present results obtained, following tentative conclusions may be drawn ; first, some ultraviolet absorbing substances diffused in larger quantity out of the irradiated cells into outer medium than out of the non-irradiated ones, although the amount of the diffused-out ultraviolet absorbing substances were comparatively small as compared with that of the total cold acid soluble ultraviolet absorbing substances in the cells. Second, the diffusion amount of these substances generally increased according to the irradiation dosages of 0.5, 1, and 2×10^5 r. Third, the nucleotides of the diffused-out substances differed in composition before and after the irradiation, specially some nucleotides, such as AMP, GMP, UDP, ATP *etc.* markedly increased in quantity after the irradiation.

We are not yet in the position to infer the mechanism of this diffusion-out phenomena of nucleotides from the cells into outer medium. The diffused-out nucleotides may originate from some intermediate metabolites of the cells or

from decomposition of some high molecular substances, such as nucleic acids. The increase in amount of the diffused-out nucleotides after the irradiation may be due to increase of permeability of plasma membrane, caused by the irradiation.[13]

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REFERENCES

(1) S. Matsuoka, *Bull. Inst. Chem. Res. Kyoto Univ.*, **43**, 17 (1965).
(2) S. Matsuoka, In preparation
(3) R. B. Hurlbert, H. Schmidt, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).
(4) W. E. Cohn and E. Volkin, *J. Biol. Chem.*, **203**, 319 (1953).
(5) W. E. Cohn, *J. Am. Chem. Soc.*, **72**, 1471 (1950). *"The Nucleic Acids,"* **1**, 211 (ed. E. Dhargaff and J. N. Davidson) (1955).
(6) G. H. Beaven, E. R. Holiday and E. A. Jonson, *"The Nucleic Acids,"* **1**, 493 (1955).
(7) D. B. Goldstein, B. J. Brown and A. Goldstein, *Biochim. Biophys. Acta*, **43**, 55 (1960).
(8) M. Drakulic and E. Kos, *Radiation Research*, **19**, 429 and 439 (1963).
(9) P. Alexander, J. T. Lett, P. Kopp and R. Itzhaki, *Radiation Research*, **14**, 363 (1961).
(10) J. A. V. Butler, *Radiation Research, Suppl.*, **1**, 403 (1959).
(11) R. E. Beyer and R. D. Kennison, *Biochim. Biophys. Acta*, **28**, 432 (1958).
(12) L. J. Cole and M. E. Ellis, *Radiation Research*, **7**, 508 (1957).
(13) V. Srb and B. Hluchovsky, *Exptl. Cell Research*, **29**, 261 (1963).